INSIDIOUS EFFECTS OF A TOXIC ESTUARINE DINOFLAGELLATE ON FISH SURVIVAL AND HUMAN HEALTH

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Abstract:

The estuarine dinoflagellate Pfiesteria piscicida gen. et sp. nov. produces exotoxin(s) that can be absorbed from water or fine aerosols. Culture filtrate (0.22 μm porosity filters, >250 toxic flagellated cells/ml) induces formation of open ulcerative sores, hemorrhaging, and death of finfish and shellfish. Human exposure to aerosols from ichthyotoxic cultures (≥ 2000 cells/ml) has been associated with narcosis, respiratory distress with asthma-like symptoms, severe stomach cramping, nausea, vomiting, and eye irritation with reddening and blurred vision (hours to days); autonomic nervous system dysfunction (localized sweating, erratic heart beat (weeks)]; central nervous system dysfunction (sudden rages and personality change (hours to days), and reversible cognitive impairment and short-term memory loss (weeks)); and chronic effects including asthma-like symptoms, exercise fatigue, and sensory symptoms (tingling or numbness in lips, hands, and feet; months to years). Elevated hepatic enzyme levels and high phosphorus excretion in one human exposure suggested hepatic and renal dysfunction (weeks); easy infection and low counts of several T-cell types may indicate immune system suppression (months to years). Pfiesteria piscicida is euryhaline and eurythermal, and in bioassays a nontoxic flagellated stage has increased under P enrichment (≥100 μg SRP/L), suggesting a stimulatory role of nutrients. Pfiesteria-like dinoflagellates have been tracked to fish kill sites in eutrophic estuaries from Delaware Bay through the Gulf Coast. Our data point to a critical need to characterize their chronic effects on human health as well as fish recruitment, disease resistance, and survival.

Article:

Within the past decade, the number of known toxic dinoflagellate species has increased worldwide from 22 to 55 [Steidinger & Baden, 1984; Steidinger, 1993 (43 species); K. Steidinger and J. M. Burkholder, unpublished data]. Although some of the newly recognized toxic dinoflagellates reflect subdivisions of known toxic species, in numerous other cases the taxa had not previously been demonstrated to produce toxins, or had not previously been known to exist. The general phenomena of increasing fish kills without apparent cause (White, 1988; Shumway, 1990) and toxic outbreaks by previously unknown or benign phytoplankton species have led to some researchers' reference to the problem as a "global epidemic" of harmful algal blooms, with dinoflagellates as major players (Smayda, 1989,.1992; Hallegraeff, 1993).

Among the estuaries associated with increasing incidence of fish kills and disease are systems in the southeastern United States such as the Pamlico and Neuse Rivers in North Carolina (Miller et al., 1990; Burkholder et al., 1993). These rivers lie within the Albemarle—Pamlico Estuarine System, which is the second largest estuary in the United States mainland in aerial extent (Copeland et al., 1984; Epperley & Ross, 1986). In that natural habitat during May 1991, the toxic dinoflagellate Pfiesteria piscicida gen. et sp. nov. (K. Steidinger, personal communication; nomen nudum, P piscimorte, P. piscimortuis) was discovered swarming in the water during a major fish kill (Burkholder et al., 1992). This organism represents a new family, genus, and species of dinoflagellate (K. Steidinger, personal communication). In the past 3.5 yr since its discovery, it has been implicated as a causative agent of major fish kills (affecting from 10^3-10^9 fish) in the Pamlico and Neuse

(Burkholder et al., 1995a).

Pfiesteria piscicida is considered a highly unusual organism for several reasons. It is the only dinoflagellate known to produce high quantities of exotoxin(s); it is also the only toxic dinoflagellate known to show direct, "deliberate" chemosensory response to targeted finfish or shellfish prey, and the only one demonstrated to have a complex life cycle with at least 19 identified stages (Burkholder & Glasgow, 1995). Most of its stages actually are animal-like amoebae (e.g., Sarcodina; Bovee and Sawyer, 1979), with size ranging from 5 to 250 μm along the major cell axis (Burkholder et al., 1995b). Several stages, including both flagellated and amoeboid forms, are toxic to finfish and shellfish (Burkholder et al., 1993). Recently, a more subtropical Pfiesteria-like species was identified (Landsberg et al., 1995); although its multiple stages have not yet been fully documented, the available information indicates that it is a close relative of P. piscicida with similar life cycle and behavior (Landsberg et al., 1995; J. Landsberg, K. Steidinger, J. M. Burkholder, and H. B. Glasgow, Jr., unpublished data).

Pfiesteria piscicida was first found as a contaminant of unknown origin in fish cultures at North Carolina State University (Smith et al., 1988). When the fish began to die, the dinoflagellate's most lethal toxic stage—a small flagellated vegetative form (Burkholder et al., 1992)—was abundant in the water, but after the fish were dead, the dinoflagellates seemed to disappear (Smith et al., 1988; Noga et al., 1993). We later determined that the small toxic flagellated vegetative cells (TFVCs) either encysted or formed amebae and sank to the bottom of the culture vessels following fish death (Burkholder et al., 1995b). The toxic stages were responding to as-yet-unidentified substance(s) in fish secreta/excreta (Burkholder et al., 1992). When threshold concentrations of these substances are detected, the dinoflagellates swim up into the water and excrete potent ecotoxin(s). The ecotoxin(s) induces similar effects on the fish whether the dinoflagellate cells are present or filtered from the water (Burkholder et al., 1992). The fish appear narcotized in that they become lethargic with poor fright response (Burkholder et al., 1993). Within minutes in active cultures with repeated fish kill history, the presence of toxin(s) is associated with stripping of the skin from the fish, producing open bleeding ulcerations (Noga et al., 1995). The dinoflagellates feed upon the freshly sloughed skin, blood, and other prey tissues; they also engulf smaller microbes during the fish-killing process (Burkholder et al., 1993; Burkholder & Glasgow, 1995; Steidinger et al., 1995). The fish lose their ability to maintain proper station, and at times gulp air at the surface in respiratory distress (Burkholder et al., 1993). Death apparently occurs by suffocation from toxin-induced muscle paralysis.

Recently, the aerosols from dilute cultures of the organism's toxic stages or direct contact with the culture medium were linked to serious health effects in laboratory workers, suggesting the potential for harmful impacts on humans as well as fish in estuarine habitat. The objectives of the present work were (1) to summarize known information for fish kills linked to this dinoflagellate in both estuaries and aquaculture facilities; (2) to examine the potential for its stimulation by inorganic nitrogen and phosphorus, two common nutrients involved in cultural eutrophication of estuaries; and (3) to assess the accumulating evidence about its role as a source of potential toxin(s).

METHODS

Culture Techniques

Culture isolates of P piscicida were collected on 23 May 1991 from the Pamlico River Estuary near channel marker 9 at the mouth of Blount Bay in Beaufort County, N.C., during an active bloom of TFVCs while approximately 1 million Atlantic menhaden (Brevoortia tyrannus Latrobe), southern flounder (Paralichthys fethostigma Jordan & Gilbert), hogchokers (Trinectes maculatus Block & Schneider), and spot (Leiostomus xanthuris Lacepede) were dying [North Carolina Department of Environment, Health & Natural Resources (NC DEHNR)—Division of Environmental Management (NC DEM), 19921. The cultures were maintained in an isolated, quarantined modular facility under 50 $\mu E\ m^{-2}\ s^{-1}$ (12:12-h light : dark cycle) in 40-L aerated, covered aquaria filled with artificial seawater at $15\%_0$ salinity (Instant Oceans sea salts in deionized water). All laboratory work in culture facilities after January 1993 was completed using full-face respirators with organic acid filters; throughout the research effort we also used disposable gloves, boots, and hair covers, and protective

clothing that was bleached to kill all stages of the dinoflagellate after use (>1% hypochlorite, or 30% bleach).

Because P piscicida requires an unidentified substance(s) in fresh fish secreta to initiate toxin production (Burkholder et al., 1992), it was necessary to maintain toxic cultures using live fish. We routinely fed the dinoflagellate tilapia (Oreochromis mossambica Peters, length 5-7 cm, rinsed thoroughly with deionized water) at a rate of 9-12 fish/d, and removed all dead fish as live fish replacements were added. The tilapia selected as the standard test species is not endemic to North Carolina but, nonetheless, is susceptible to the dinoflagellate's toxin(s). This species offered the advantages of constant availability, wide salinity tolerance, and certainty of no prior contamination by local estuarine populations of P piscicida. Prior to introduction of fish, aquaria were prepared by filling with 15‰ salinity water. The water was continually filtered and aerated, and each aquarium was maintained 14 d before adding three tilapia. Fish were acclimated for 7 d to ensure viability, and were fed daily with several flakes of Tetra Maria food. The aquarium cultures also contained occasional small flagellates (e.g., Tetraselmis), blue-green algae (Lyngbya, Gloeothece), and protozoan ciliates (Saprophilus, Microthorax, Styolonichia).

Fish Kills and Bioassays to Confirm Toxicity

From May 1991 to November 1993, we checked fresh and preserved surface water samples (preservative, acidic Lugol's solution; Vollenweider, 1974) for the presence of P piscicida at in-progress fish kills in estuaries including the Pamlico and Neuse estuaries (including several sites near a phosphate mine or municipal wastewater discharges); a nutrient-enriched coastal site (Taylors Creek, near municipal wastewater discharge and a menhaden processing plant; Burkholder et al., 1995a); two relatively nutrient-poor coastal sites (Wrightsville Beach and Topsail Beach); and aquaculture facilities with fish kills/disease (e.g., National Marine Fisheries Service, Beaufort, N.C., and the Department of Zoology, North Carolina State University).

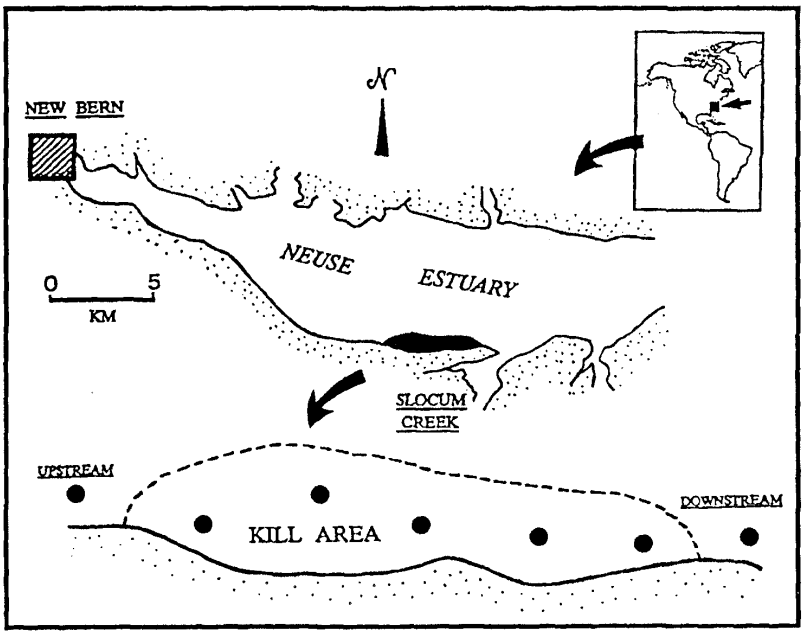

FIGURE 1. Location and extent of the Atlantic menhaden kill area sampled in the Neuse River Estuary at *2000 h on 30 July 1993 (dots = sampling sites). A brownish surface foam or "slick," having the appearance of crude oil, covered most of the kill area from 1500 to 2200 h. A local citizen reported that he had noticed the slick forming while boating on the river on 29 July. He described menhaden in the area on that date as lethargic, swimming slowly in wide circles with disoriented, abnormal behavior (e.g., swimming upside down) and apparent loss of fright response. The slick was probably formed by oils and other excreta/secreta from the affected menhaden, and it was found to contain P. piscicida in high abundance.*

At most of the kills, surface water was sampled from what was estimated to be a central location among dead/dying fish. One menhaden kill (30-31 July 1993, Neuse Estuary) was sampled to obtain a more detailed profile of environmental conditions and abundance of flagellated, amoeboid, and encysted stages of P. piscicida. This kill lasted 21 h (from 1500 h on 30 July to 1100 h on 31 July). Conditions were calm throughout the first day; strong winds developed (15-20 knots from the northwest), however, at approximately 2300 h on 30 July, and the kill was completely dispersed by 1100 h on 31 July. The kill covered a 5-km length of the Neuse from

above the town of Carolina Pines near Harmers Beach downstream to ~0.4 km above the entry point of Slocum Creek; in width it extended from the river's south shore out 300-450 m (Figure 1). The affected fish were either in shallow water (depth ≤1 m, representing most of the kill area), or were distributed in the upper 1.0 m of the water column.

A Hydrolab sensor (model H20, with surveyor 3 datalogger) was used to measure temperature, salinity, pH, and dissolved oxygen from the surface, 0.5-m, and 1.0-m depths at five sites positioned evenly throughout the kill area. One site 300 m upstream and one site 300 m downstream from the outermost extent of dead/dying fish were sampled similarly. Nutrients were analyzed from water samples collected at depth 0.5 m. Total phosphorus was measured after acid persulfate digestion (Parsons et al., 1985); soluble reactive phosphate (SRP) was analyzed using the same procedure, but without persulfate digestion and after filtering the samples (cleaned 0.22 µm pore-size Millipore GS filters). Nitrate was determined on a Technicon auto-analyzer (model II) following copper—cadmium reduction (Parsons et al., 1985). Ammonium was analyzed with the Solorzano method (Parsons et al., 1985), using modifications of Burkholder and Sheath (1985) for immediate preservation with phenol. Pfiesteria piscicida stages from depth 0.5 m were quantified with an Olympus IMT-2 inverted microscope (600×, phase optics), using the Utermohl technique as in Burkholder and Wetzel (1989).

Aquarium bioassays with field water samples and test tilapia were used to discern the small TFVCs from other co-occurring, nontoxic estuarine dinoflagellates that are similar in appearance under light microscopy. Bioassays were also used to determine whether populations of P piscicida that had been collected at kill sites exhibited toxic activity. The bioassays were maintained under similar conditions as those used to maintain stock dinoflagellate cultures, and toxic stages were quantified using the Utermöhl technique. Stage identifications in water from both fish kills and bioassays were confirmed using scanning electron microscopy (Burkholder & Glasgow, 1995).

Response to Nitrogen and Phosphorus Enrichments

Experiments to test the effects of inorganic nitrate, ammonium, and phosphate enrichments on growth of P piscicida were conducted for 4 d as batch cultures maintained in gently aerated 500-m1 Erlenmeyer flasks with artificial seawater at 15‰ (Instant Ocean salts with background TP, NO_3-N, and NH_4^+N each ~5 p=µg/L). We gently concentrated dinoflagellate cells and separated them from enriched water with fish excreta by drop-filtering (as slow gravity filtration) 200 ml of stock culture (with ~1000 flagellated cells/rill) through Whatman GFC glass fiber filters. The filtering procedure induced most TFVCs and planozygotes to form cysts or amoebae, but many gametes retained their flagella and reverted to asexual zoospores (Burkholder & Glasgow, 1995). One wet filter with cells was transferred to each flask and, after acclimation overnight, nutrient treatments were initiated in triplicate.

In testing the response of P piscicida to inorganic nitrogen and phosphorus enrichments, we compared growth in controls (without nutrient additions, designated as 5 µg/L) and treatments, which were established by adding an initial spike of phosphate, nitrate, or ammonium to attain concentrations of 25, 100, or 1000 µg PO_4^{3-}P, NO_3^-N, or NH_4^+N/L. A 50-m1 sample was collected from each flask after 4 d, and was preserved and analyzed for abundance of dinoflagellate stages. The selected nutrient levels were within the range reported for the lower Neuse River (Paerl et al., 1990; Christian et al., 1991: PO_4^{3-}P typically at 60 µg/L with a maximum at 250 µg/L; NO_3^-N at 15-20 µg/L except for increases up to 170 µg/L during 6- to 8-wk periods of precipitation and runoff; NH_4^+N at 30 µg/L, with increases up to ~140 µg/L during precipitation/runoff events). In the study area of the Pamlico Estuary, nitrogen was at comparable levels as in the Neuse, but phosphate was ~300 µg/L to >640 µg/L during summer low-flow periods prior to 1993. The Pamlico differs from the Neuse in that it lies on a geological phosphorus formation. Adjacent to the lower Pamlico, Texasgulf Chemical Company operates the world's largest phosphorus mine and discharged ~2500 metric tons of P daily from the late 1960s to 1992 (Steel, 1991; NC DEHNR, unpublished records). This point source was reduced by >90% beginning in fall 1992, but it represented 50% of the total phosphorus loading to the lower Pamlico Estuary during the previous ~25-yr period (Steel, 1991; NC DEHNR, 1990, unpublished records). Following the imposed P reductions from Texasgulf, phosphate in the waters of the Pamlico sampling area generally has ranged from ~50 to 120 µg PO_4^{3-}

P/L, although the sediments are assumed to remain highly phosphate enriched (NC DEHNR, unpublished records; J. M. Burkholder & H. B. Glasgow, Jr., unpublished data).

Assessment of Human Exposures

People working without protective respirators near P piscicida cultures reported a complex of symptoms that suggested exposure to sensitizing and/or toxic substances associated with these cultures. We examined three cases with serious illnesses after laboratory exposure in which medical investigations were undertaken, as well as a group of cases who reported transient symptomatology. To preserve confidentiality, reference to exact age or gender was excluded. Moreover, various commercial and recreational fishermen who frequent sites of major toxic activity by this dinoflagellate anecdotally have reported skin lesions on hands, arms, and legs with poor healing, and sometimes episodes of "foggy memory" or apparent reversible short-term memory loss, after fishing for extended periods (hours to days) in these areas to several of the authors. Information is included from co-author D. E. Schmechel's interview with one such person who described a transient neurological syndrome and skin lesions after immersion exposure.

TABLE 1. Environmental Conditions and Fish Affected at Kills Linked to *Pfiesteria piscicida* in North Carolina Estuaries, Coastal Waters, and Aquaculture Facilities During 1991–1993

Habitat	Temperature (°C)	Salinity (‰)	Nutrients (μg/L)	*Pfiesteria* (cells/ml)[a]	Fish affected[a]
Estuaries					
Neuse	28 ± 1	10 ± 1	TP 200 ± 35 TKN 1015 ± 295	810 ± 100	Atlantic menhaden, blue crab, catfish, mullet, spot, white perch (>1 × 10^9)
Pamlico	29 ± 1	7 ± 1	TP 290 ± 4 TKN 580 ± 45	4300 ± 2510	Atlantic menhaden, American eel, blue crab, croaker, hog-choker, southern flounder, spot (>2 × 10^6)
Coastal					
Near WWTP, inner channel	15	30	NA	11,960	Southern flounder, American eel, sheepshead (2 × 10^4)
Open Atlantic	13 ± 4	35	NA	1400[a]	Atlantic menhaden (>6 × 10^3)
Aquaculture	17 ± 2	22 ± 3	NA	820 ± 340	Atlantic menhaden, bay scallop, hybrid striped bass, littleneck clam, naked goby, sheepshead, southern flounder, tilapia, white perch (>4 × 10^4; >$100,00 loss)

Note. Data are given as the mean ± 1 standard error (SE); $n = 7$ (Neuse; including 5 major kills defined as affecting ≥10^3 fish, and 2 minor kills affecting several hundred fish); 10 (Pamlico; including 9 major kills); 1 (major kill in nutrient-enriched coastal waters, Taylors Creek downstream from a wastewater treatment plant [WWTP]); 2 in relatively nutrient-poor coastal waters (major kills, Wrightsville Beach and Topsail Beach); and 9 (aquaculture facilities including both indoor tanks and outdoor ponds). Nutrient data were not available (NA) for the coastal waters or aquaculture systems.

[a] "Cells/ml" data include both flagellated and amoeboid stages except for 1991, in which amoeboid stages were not recognized as part of the life cycle. The large error bar in Pamlico dinoflagellate abundance reflects interannual variability as well as other factors, such as the time lag between the kill and sampling. "Fish affected" data include the total number of fish known to have been involved in all kills linked to *P. piscicida* and the total cost for the reported aquaculture kills. Note that of the nonenriched kill sites, the Wrightsville Beach area was sampled 2 d after the kill, and contained mostly amoeboid forms along with ~50 TFVCs/ml. The sample was not considered among the quantitative information used to construct this table, because of the substantial time lapse between the kill and the sample effort. Scientific names for fish not referenced in the text are *Icatalurus punctatus* L. (channel catfish), *Mugil cephalus* L. (striped mullet), *Morone americana* Gmelin (white perch), *Anguilla rostrata* Lesueur (American eel), *Micropogonias undulatus* L. (Atlantic croaker), *Archosargus probatocephalus* Walbaum (sheepshead), *Gobiosoma bosc* Lacepede (naked goby), and *Oreochromis aureus* Steindachner and *Tilapia nolotica* L. (other tilapia species).

RESULTS

Fish Kills

Among the 21 field kills in 1991-1993 with documented toxic activity by P. piscicida, 4 were coastal and the remainder occurred in mesohaline estuaries (Table 1). In the two major estuaries where nutrient data were available, total phosphorus and total Kjeldahl nitrogen averaged ≥200 μg/L and ≥580 μg/L, respectively (Table 1), indicating that toxic outbreaks were most frequent in nutrient-enriched waters (Rudek et al., 1991; Jaworski et al., 1992; Dennison et al., 1993; Mallin, 1994). About half of the kills occurred near wastewater treatment facilities, fish processing plants, domestic animal operations, or phosphate mining. TFVCs were implicated in 17 of 33 major fish kills (involving 10^3 to ~10^9 fish) on record for the Pamlico and Neuse estuaries during a 3-

yr period [9 of 15 in 1991, 4 of 8 in 1992, and 4 of 10 in 1993 (but note—samples from only 4 of the 10 kills in 1993 were available); K. Miller, NC DEM and NC DMF, unpublished fish kill records; Burkholder et al., 1995a]. Hence, based on 1991-1993 NC DEHNR data in combination with our sampling effort and confirming bioassays, R piscicida was implicated as the causative agent of 50 ± 8% [mean ± 1 standard error (SE)] of the major fish kills that occurred annually in two of North Carolina's major estuaries.

Although the estuarine/coastal kills associated with this pathogen have included a variety of finfish and shellfish (Table 1), many have involved schooling fish such as Atlantic menhaden that occur in dense populations and produce copious secreta. Brownish foam frequently developed during these kills in areas with high abundance of floating fish and dinoflagellates. In the menhaden kill that was characterized in more detail, measurements completed at 2000 h under calm conditions prior to the onset of moderate winds (25 knots beginning at ~2200 h) indicated that physical/chemical conditions were comparable at sites within versus outside the affected area (Table 2). The water was phosphorus replete (~150 μg SRP/L) with low inorganic nitrogen (~10 μg NO_3^-N and 20 μg NH_4^+NUL). During this calm period while the kill was in progress, abundances of TFVCs and gametes in surface waters were significantly higher within the affected area than in upstream or downstream locations without dead fish (Table 2). Planozygotes and cysts were rare in the affected area, and amoeboid stages were uncommon (20 ± 5 cells/nil). Slicks, or areas of brownish foam at the water surface in the kill areas, provided an interesting contrast to these general trends. The foam contained intermediate but lethal densities of TFVCs (mean ± SE, 560 ± 20 cells/ml, n = 3; range from >300 to >2000/m1) and extremely high abundance of gametes (mean 1.09 ± 0.02 × 10^5 cells/ml, n = 3; range 10^5 to 10^6 cells/ml), many of which were found transforming to small amoebae within gelatinous masses. Larger amoeboid stages were also more common in the foam than in the surrounding surface water, and levels of phosphorus, ammonium, and suspended solids (SS) were much higher (e.g., nearly 1 mg TP/L, >1.7 mg NH_4^+N/L, and >880 mg SS/L). We later observed this foam being widely broadcast by prevailing winds.

TABLE 2. Environmental Conditions and Abundance of *Pfiesteria piscicida* in the Vicinity of the Atlantic Menhaden Kill on 30 July 1993 in Surface Waters of the Mesohaline Neuse Estuary (Depth 0.5 m)

Variable	Kill area	Slick[a]	Outside
Environmental			
Temperature (°C)[b]	29 ± 0	30 ± 1	30 ± 0
Salinity (‰)[b]	15 ± 1	NA	13 ± 2
pH[b]	8.1 ± 0.0	NA	8.4 ± 0.4
D.O. (mg/L)[b]	7.3 ± 0.1	NA	9.7 ± 3.0
SS (mg/L)	48 ± 27	≥880**	35 ± 3
TP (μg/L)	319 ± 16	≥950**	303 ± 28
SRP (μg/L)	152 ± 5	≥300**	158 ± 3
NO_3^-N (μg/L)	10 ± 3	NA	8 ± 3
NH_4^+N (μg/L)	20 ± 3	≥1700**	15 ± 0
Pfiesteria piscicida			
TFVCs	600 ± 100*	560 ± 20*	110 ± 20
Gametes	150 ± 10*	108,620 ± 1380**	60 ± 20
Planozygotes	0	0	<5 (2 ± 2)
Cysts	<5 (3 ± 2)	0	0
Amoebae	20 ± 5	70 ± 10*	<5 (4 ± 1)

Note. Asterisks indicate significant differences at the $*p < .05$ or $**p < .01$ levels (one-way analysis of variance followed by comparison of means using Fisher's protected least significant difference test, with a comparison-wise error rate; $\alpha = 0.05$; SAS, Inc., 1987).

[a]Nutrients and suspended solids were highly variable in the slick area; for purposes of comparison, the lowest values are given. NA, not available.

[b]Among kill sites, temperature and salinity were uniform at 0.0, 0.5, and 1.0 m depths; pH decreased with depth to 8.0 ± 0.2 at 1.0 m; and dissolved oxygen (D.O.) was 6.8 ± 0.2 mg/L at 0.5 m and 6.5 ± 0.3 mg/L at 1.0 m. Outside the kill sites at depth 1.0 m, temperature and pH decreased (relative to surface conditions) to 28 ± 0°C and 8.1 ± 0, respectively; salinity increased to 15 ± 0‰, and D.O. decreased to 6.8 ± 0 mg/L.

During the 1991-1993 study period, fish kills linked to R piscicida occurred across broad temperature and salinity regimes (10-33°C and 2-35%, respectively), with most frequent toxic outbreaks at ~11-15% and temperatures >26°C (Table 1). Aquaculture facilities near known kill sites, such as the National Marine Fisheries Service (Beaufort, N.C.), also supported toxic populations of the dinoflagellate during winter months (Table 1), sometimes in 0‰ water with appreciable calcium (total hardness = 20 mg/L). The data suggest that toxic stages are relatively inactive during cold periods but that lethal activity can resume when the water is warmed, regardless of season.

Response to Inorganic N and P Enrichments

After 4 d in batch culture, zoospores resembling I? piscicida gametes (but frequently transforming to small amoebae and not observed to fuse, having lost sexual potential; Burkholder & Glasgow, 1995) were strongly stimulated by phosphate enrichment at levels ≥100 µg SRP/L (Figure 2), typical of conditions in the Pamlico and Neuse estuaries (Rudek et al., 1991; Stanley, 1992; Steel & Spence, 1995). Low nitrate enrichment (25 µg NO_3^-N/L) mildly stimulated growth relative to that in controls without nitrate enrichment, whereas ammonium treatments negligibly affected cell abundance.

Three Cases of Human Laboratory Exposure to Pfiesteria Cultures

Case A. A marine scientist (less than 40 yr of age) was working with dilute cultures (<2 × 10^3 cells/ml) of the dinoflagellate's toxic flagellated stages, cultured by active fish killing. Exposures included routine direct contact with hands and arms, and potential aerosols and inhalational exposures from being near loosely covered or open aquaria. Over 2-3 mo, this person experienced subacute onset of sensory disturbance, experienced as numbness and tingling in hands and feet, and difficulty walking with problems knowing that his feet were present (Table 3). There were no hot/cold reversal phenomena when mild to moderate myalgia was present. Skin lesions developed on hands and arms, with pustular blisters that disappeared and then reappeared. Fogginess and problems with mental concentration were also experienced.

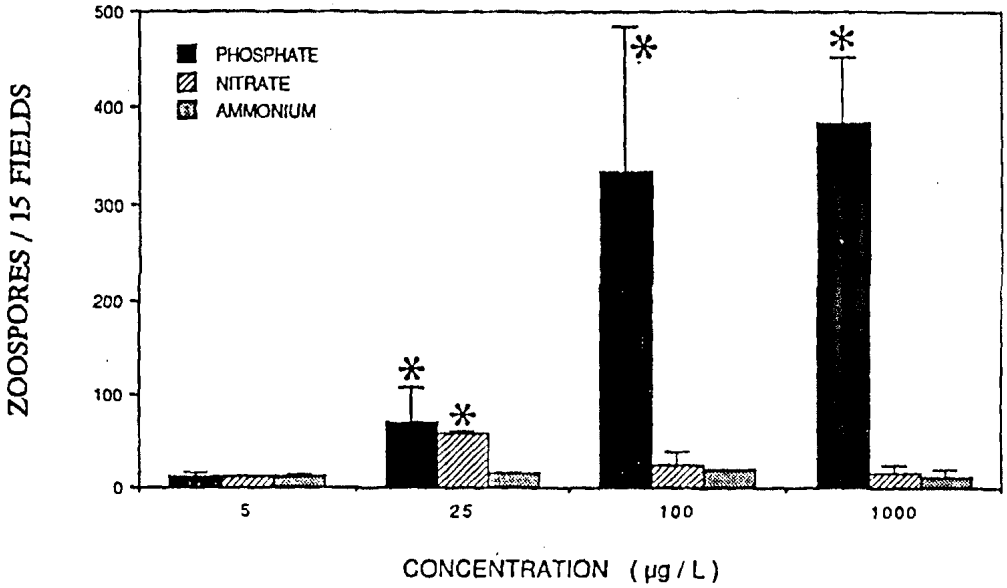

FIGURE 2. *Response of P. piscicida* amoeboid zoospores (reverted from gametes, and transformed/transforming into amoebae that were taken from actively killing cultures) to gradients of phosphate (PO_4^{-3}P), nitrate (NO_3^-N), and ammonium (NH_4^+N) enrichment after 4 d in batch cultures without finfish (means ± SE, $n = 3$). Note: The 5 µg/L concentration (background nutrient levels in the Instant Ocean media) represented the response in controls without nutrient additions. Asterisks indicate significant differences (tests for homogeneity of variance [Hartley's test; Gill, 1978], followed by one-way ANOVAs and comparison of means using Fisher's protected least significant difference test, with a comparison-wise error rate; $\alpha = 0.05$ [SAS, Inc., 1987; Day & Quinn, 1989]). Also note that in three of four experiments, we were able to repeat these results. The fourth experiment involved a culture inoculum that had not been exposed to live fish for 5 d, and the culture did not show P stimulation. These results suggest that the response of this animal-like dinoflagellate to nutrient enrichment depends, at least in part, on its recent food supplies, and that nutrient controls on growth of various stages likely are more complex than typically considered for plant-like dinoflagellates and other algae.

The scientist presented to medical attention for these problems. Differential diagnoses mentioned in the received medical records included Lyme disease, toxic or autoimmune peripheral neuropathy, and possibly multiple sclerosis. Neurological exam was reported as essentially normal and nonfocal, except for decreased ankle reflexes. Medical evaluation included routine bloodwork, urine collections, spinal tap, and electromyography/nerve conduction studies (EMG/NCV), but not neuropsychological testing. Specialty consultations included two neurologists, an infectious disease specialist, and a dermatologist. The studies revealed essentially normal EMG/NCV (mild electromyographic changes with normal sensory and motor nerve conduction velocities consistent with minimal EMG evidence for motor axonopathy and no evidence for Guillain-Barrd syndrome); normal urinary excretion of arsenic, lead, mercury, and cadmium; and moderate elevation of spinal fluid protein and immunoglobulin G (IgG) without oligoclonal bands or cells. All bloodwork was negative including Lyme disease titers, except for marked elevation of creatine phosphate kinase (CPK). Apparent diagnosis was transient neuropathic syndrome, possibly postinfectious, and with the patient improving, no treatment or followup occurred. Fearing additional exposure to cultures, the scientist immediately ceased working with the dineflagellate, but continued to experience mild problems in concentration for several months, according to colleagues. The scientist does not now recall mental difficulties. Over a 1-to 2-yr period, most symptoms resolved. The scientist has described residual mild sensory symptoms after exercise in the ensuing years. The scientist remains actively employed in the academic community, and further followup medical evaluation is planned.

TABLE 3. Symptoms in Laboratory Exposures to Toxic Cultures of *Pfiesteria piscicida*

Symptom	Case A	Case B	Case C	Others ($n = 7$)
Extremity paresthesias	+	+	–	–
Circumoral paresthesias	?	+	–	–
Paradoxical paresthesias	–	–	–	–
Arthralgia	–	–	+	2
Myalgia	+	–	–	2
Diarrhea	–	–	–	–
Asthenia	+	+	+	2
Headache	–	+	+	–
Pruritus	–	–	–	–
Nausea	–	+	+	1
Abdominal pain	–	+	+	1
Vomiting	–	+	–	–
Perspiration	–	+	–	–
Tearing/eye irritation	–	+	+	4
Dyspnea/respiratory problems	–	+	+	3
Paresis	–	–	–	–
Memory problems	+	+	+	1
Emotional changes	+	+	+	–
Skin lesions	+	+	+	–

Note. Adapted from Swift and Swift (1993), with rank order following that of ciguatera poisoning.

Case B. A marine scientist (less than 40 yr of age) accepted responsibility for culture-tending duties for a technician who feared working with cultures after stomach cramping in the presence of dilute cultures within an environmental chamber. There was no significant previous medical illness or symptomatology prior to exposures; this person reported occasional moderate use of social ethanol without other substance use or medications. In April 1992 the scientist was exposed to an aerosol of active culture water when cleaning the chamber and aquaria that had been emptied of toxic culture (previously ~5000 toxic cells/ml), using a hose without ventilator or gloves. Sudden onset of rapid thought and giddiness was noted, while sense of distance was impaired and movement was slowed (Table 3). After the scientist ceased the activity and left the chamber, recovery occurred within ~20 min. During November 1992, this person was exposed to aerosols from a large volume of active culture water (~2000 toxic cells/ml) that the culturist in charge had allowed to evaporate. Within 10 min while attempting to exchange dead with live fish, the scientist experienced burning eyes, disorientation with a sense of depersonalization and of being compelled to continue, followed by nausea and

vomiting (Table 3). This person was nearly overcome and crawled out of the environmental chamber, recovering after ~1 h; after this episode, the scientist did not reenter the environmental chamber.

During November 1992 to January 1993, the scientist worked without a protective respirator for 2 h/d in a larger, better aerated room with toxic cultures (500-90,000 toxic cells/ml) but still experienced chest tightness and shortness of breath, which slowly subsided several hours after leaving. Because of concern over aerosol exposures, further precautions, including use of respirators with organic acid filters, were initiated. In late summer 1993 this person began to work in a third isolated facility that had been designed for more than 12 air exchanges/h, but received 8 wk of low-level exposure to toxic cultures (twelve 7-L aquaria, one 14-L aquarium, and one 28-1 aquarium, all tightly covered except when sampling or changing out fish; culture densities <2000 toxic cells/ml for 5 wk, and <5000 toxic cells/ml for 3 subsequent weeks). The additional chronic exposure likely resulted from improper construction of ventilation for the facility by an outside contractor, so that air from the culture room vented into "safe" office space where this scientist frequently stayed during the day. Subacute deterioration ensued with changes in personality, irritability, loss of judgment, poor memory, and degradation of performance noted by other laboratory staff. The behavioral changes were attributed, at first, to emotional problems, common illnesses such as colds or flu, or stress. The scientist also developed skin lesions on hands and forearms, and difficulty in sleeping. Subsequent intensive work with dilute toxic cultures over a 2-d period in November 1993 was associated with significant deterioration with impaired speech and language, difficulty in spatial orientation while driving, and marked personality change noted by the laboratory supervisor and the spouse. In early December 1993, the scientist was seen by an internist who recommended cessation of exposure.

Within several days after cessation of exposure, the patient markedly improved, although with significant residual symptomatology indicated from neurological evaluation. The scientist complained of short-term memory deficits, difficulty reading and speaking, spatial disorientation, severe throbbing headaches, episodic rage (noted by the spouse), and emotional lability; labile pulse and blood pressure without syncope accompanied by markedly excessive sweating of hands, underarms, feet, and face; skin infections with folliculitis and recurring pustules developing into open sores over chest and arms; and difficulty in sleeping.

Extensive medical testing was performed because of the severity of symptoms and the uncertain prognosis. Physical examination showed normal vital signs, markedly excessive sweating asymmetrically on the body in an air-conditioned room, and numerous pustules and open sores over the chest and forearms that had not responded to treatment with antibiotics. Observations and neurological examination supported symptoms of irritability, decreased fluency with hesitation in word choice and scanning-like speech, slowed and impaired reading, and inability to perform simple calculations or recite months backward. The neurological examination was otherwise normal (e.g., in sensation, strength, and gait) except for slightly pendular reflexes, mild dysdiachokinesia, and problems with heel-to-shin and finger-to-nose maneuvers. Full bloodwork for reversible etiologies of memory problems was normal, except for moderate and variable elevation of SGPT and borderline-low serum phosphorus. No other tests of kidney or hepatic function were abnormal. A brain magnetic resonance imaging (MRI) scan was normal except for minimal (borderline-significant) changes in the left hippocampus. A fluerode-oxyglucose positron emission tomography (PET) scan was also normal. EMG/NCV testing showed no evidence of peripheral neuropathy or autonomic neuropathy. Visual- and brainstem-evoked potentials were normal, as was an electroencephalogram. Full neuropsychological testing supported a definite organic syndrome with amnestic syndrome, involving verbal more than visual modalities. Definite clinical improvement occurred over several weeks, with improvement and normal memory documented 2 mo after the last toxin exposure. Since improvement rapidly occurred, there was no specific treatment other than recommendation of cessation of exposure to toxic cultures, and of driving and work-related activities for safety reasons and to minimize stress over the 2-mo recovery period.

After 11 mo without exposure, the scientist's physical and neurological examinations were normal. However, for 8 mo residual symptomatology included asthenia, sudden extreme irritability, and "foggy" memory episodes lasting about 12 h, which began several hours after strenuous exercise that formerly was routine in daily

activities (e.g., running; no clinical evidence for abnormal level of consciousness), with possible heat intolerance, ethanol-induced severe headaches similar to the original headache syndrome, occasional episodes of paresthesias of hands and fingers, and perioral numbness. All of these effects increased following vigorous exercise. After 12 mo without exposure, the scientist continued to experience moderate myalgia and occasional erratic mood swings and sensory symptomatology (e.g., numb fingertips) after exercise, but not foggy memory. Further medical followup and evaluation are planned.

Case C. A marine scientist (less than 40 yr of age) shared some culture duties (e.g., changing out dead fish and maintaining/sampling experiments) for 3 mo working mostly with dilute cultures (<5000 toxic cells/rill), followed by an acute short-term exposure to concentrated cultures (90,000 toxic cells/ml). There were no previous medical problems prior to toxic culture exposures except for mild asthma and a history of recurrent bronchitis following colds, once or twice annually. On the same date in January 1993 as Case B, after working with contaminated cultures for ~4 h, the scientist experienced the onset of burning eyes, nausea with stomach cramping, and disorientation with abnormal mental state (Table 3). This person experienced severe headaches and arthralgia, as well as depersonalization, which was described as a feeling of watching him-/herself, with a sensation of being compelled to continue the work, rubbing burning eyes with wet culture-contaminated gloves, and lacking ability to recognize that "something was wrong."

The scientist eventually left the culture room when the work was finished, and was driven home by a colleague. Eight days of acute difficulty with short-term memory followed, with some visual retention but cognitive impairment. This person described frustration and fear at being unable to dial a telephone number unless holding the written sequence adjacent to the telephone and referring to the note for each number in the sequence; conversations were not possible because of failure to remember initial parts of sentences; writing was not possible because individual words were recognized, but their meaning in sequence as collective thoughts could not be grasped. In the 2-yr period after this exposure, the scientist has experienced chronic respiratory distress and recurrent respiratory infections with asthmatic bronchitis. There were no noted paresthesias or abnormal perspiring, nor apparent abnormal response to alcohol. As in Case B, strenuous exercise (running 6 km daily) had been consistent in this person's daily routine for more than 3 yr prior to the January 1993 exposure. Since that time, however, attempts to run 2 km/d or more have exacerbated the problem, inducing shortness of breath, asthma, chronic colds, and respiratory infections including repeated incidence of pneumonia. In addition, T-cell counts remain somewhat low, suggesting immune system dysfunction. Further medical followup and evaluation are planned.

Other Laboratory Exposures. Seven other laboratory personnel or marine scientists have reported symptoms either while working with P piscicida cultures (n = 5) or when occupying offices near the culture area (n = 2; Table 3). In all cases, symptoms occurred when in close proximity to toxic cultures, and terminated within a few hours to several days after exposures ceased. The time-locking of symptomatology convinced all that the cultures were responsible. In three cases, exposure apparently did not involve direct contact with cultures, and may have represented aerosol or inhalational exposures. One of the seven cases may represent modeling, since dizziness was only experienced while witnessing another person with eye irritation. Two cases subsequently used face masks/ventilators, and symptoms ceased after this precaution was taken.

Possible Cases in Fishermen of Either Sex. Common sense wisdom in areas of North Carolina with frequent estuarine fish kills is not to swim during kills. Apparently many coastal residents follow this practice, although on some occasions people have been observed in activities such as collecting freshly dead fish for consumption, or water skiing through kill sites. Commercial fishermen who work areas where fish are caught with ulcerative mycoses speak of "fish poisoning" and rinse their hands with bleach after sorting fish with epidermal ulcerations. Anecdotal evidence has been obtained that following prolonged contact with the water in areas where toxic outbreaks of the dinoflagellate have been documented, some fishermen have experienced persistent skin lesions on hands, arms, and legs with poor healing, and sometimes episodes of fogginess. These lesions apparently have not responded to routinely prescribed antibiotics. While they commonly develop when initiated as cuts from finfish or shellfish spines, lesions of similar appearance also have been reported without

involvement of these prior abrasions. Bleach rinse, known to kill the dinoflagellate in laboratory practices (Burkholder et al., 1993), is used by fishermen because it is thought to prevent skin lesions.

Co-author D. E. Schmechel interviewed a younger recreational fisherman with a transient neurological syndrome and skin lesions after immersion exposure in an area (unknown to this person) of toxic activity by R as follows: In August 1992, the fisherman (less than 40 yr old) spent a long day fishing for shrimp near the home of a relative, in an area of the Pamlico Estuary with history of fish kills. By the afternoon the fisherman noticed that sores had developed on his/her forearms and hands. Prior cuts from shrimp spines preceded some of the sores. Soon afterward, this person felt unwell with slight nausea, fogginess, and altered mental state. Some of the shrimp were consumed that evening after onset of symptoms. There was no prior or concurrent alcohol use or substance abuse. Intending to return home by car the next day (~2-h drive), the fisherman arose but felt "too out of it" to begin the journey and stayed with a relative for 2 d before attempting the drive. This person continued to have feelings of impaired memory and performance during that time, and even stopped in mid-journey for one more evening at a friend's home because of concern about impaired driving ability. The mental fogginess receded after several days, without recurrence or apparent residual symptoms. The skin lesions on the fisherman's forearms slowly healed over the following week.

DISCUSSION AND CONCLUSIONS

Since the discovery of P piscicida in the Albermarle—Pamlico (Burkholder et al., 1992), accumulating data indicate that this dinoflagellate is a significant source of environmental stress in estuarine ecosystems. It has been implicated as the causative agent of ~50% of the major fish kills annually in these estuaries, and is most prevalent in locations influenced by anthropogenic nutrient loading from municipal wastewater, phosphate mining, fish processing plant discharge, and other sources (Burkholder et al., 1995a). Various finfish and shellfish species indigenous to the study area were assayed for susceptibility to P. piscicida toxin(s), and 100% mortality occurred for all after short-term exposure (minutes to hours). Summarized data on fish kills in estuaries, coastal areas, and aquaculture facilities indicate that thousands to millions of fish are affected in many of the kills, suggesting potential monetary loss of hundreds of thousands of dollars to the fishing industry (National Oceanic & Atmospheric Administration, 1992).

The common occurrence of fish with ulcerations in the Pamlico and Neuse estuaries may indicate that wild populations periodically receive chronic exposure to sublethal levels of the dinoflagellate's toxin(s). Fish exposed to filtrate (0.22 μm porosity Nuclepore filters) from cultures of P. piscicida have shown an array of behavioral and pathological signs (Smith et al., 1988; Burkholder et al., 1993; Noga et al., 1993). Behavioral responses documented thus far include darkening of epidermis, erratic swimming, decreased respiration, and convulsions; pathologies include skin lesions or ulcerations (Noga et al., 1995), and apparent muscle paralysis indicating central and/or peripheral nervous system damage. Shell disease has also been observed in blue crabs (Callinectes sapidus Rathbun) exposed to the TFVCs (H. B. Glasgow, Jr., & J. M. Burkholder, unpublished data), and is common for wild crab populations in major field kill sites such as the lower Pamlico Estuary (Noga et al., 1989).

Epidermal lesions, sometimes called "red sores," are common among finfish especially in the Pamlico Estuary (Levine et al., 1990; Noga et at, 1991); for example, in monthly sampling, 30-98% of adult fish from all species sampled in the lower Pamlico had developed obvious gaping lesions during May to early July 1994 (n = 700; Burkholder et al., 1995a). Ulcerations in finfish have been linked to factors such as pesticides, polychlorinated biphenyls (PCBs), and fungal pathogens (Snieszko & Axelrod, 1976). Examination of affected tissue in fish exposed to toxic cultures of P piscicida revealed that opportunistic bacterial and fungal pathogens had colonized from the lesion surface down toward the base but were not found near or at the base, suggesting that some other agent created the lesion (Noga & Dykstra, 1986; Noga et al., 1995). While pollutants or other pathogens may have formed the lesions observed in the fish cultures exposed to this dinoflagellate, the toxin(s) of P piscicida recently was demonstrated to be the causative agent of ulcerative "mycosis" in Atlantic menhaden (Noga et al., 1995).

Toxic outbreaks of P piscicida have occurred across broad gradients of salinity and temperature ranging over 0-35‰ and 10-33°C, respectively (Burkholder et al., 1995a). Laboratory and field data indicate that this organism is a warm-temperate estuarine species, with optimum salinity for toxicity at 15‰ and outbreaks most frequent in waters ≥26°C. During fish kills surface waters contain hundreds to thousands of TFVCs as well as gametes, toxic planozygotes, and occasional amebae. Brown surface foam that develops during many of these kills can contain high gamete abundance, suggesting that wind-distributed foam could represent an effective dispersal mechanism for this toxic dinoflagellate.

Mid-range salinities, warm temperatures, moderate winds, and nutrient-enriched waters characterize the Neuse and Pamlico estuaries (Steel & Spence, 1995). The Pamlico, in particular, is phosphate enriched and appears to provide highly favorable habitat for R piscicida (Burkholder et al., 1995a). Our laboratory bioassay data suggest that nutrient loading may play an important role in supporting a water-column inoculum of nontoxic stages (e.g., zoospores from reverted gametes), which can develop the capacity for toxin production when they detect secreta from live fish (Burkholder & Glasgow, 1995). The eurythermal, euryhaline characteristics of this dinoflagellate suggest that it is widespread in nutrient-enriched, warm temperate estuaries. Both known Pfiesteria-like species have been tracked to aquaculture facilities (Burkholder et al., 1995a; Landsberg et al., 1995). Moreover, in laboratory bioassays P. piscicida has proven lethal to all 19 species of both native and exotic finfish and shellfish tested in culture, including commercially valuable species such as Atlantic menhaden, red drum (Sciaenops ocellatus L.), southern flounder, striped bass (Morone saxatilis Walbaum), hybrid striped bass (Morone saxatilis × Morone chrysops Rafinesque), blue crabs, bay scallops (Aequipecten irradians Lamarck), and littleneck clams (northern quahog, Mercenaria mercinaria Linne). These data suggest the potential for transport of sublethal densities of this organism with cultured fish as another means of dispersal.

Molecular-level controls on toxin production by P. piscicida need to be determined. Other algal toxins have been implicated in development of malignant tumors, and at low levels some of these toxins may be of use in treating cancer and Alzheimer's disease (Carmichael, 1994). Application of sublethal level(s) of toxin(s) from this dinoflagellate may afford similar benefits, and will enable insights about molecular mechanisms that control neurological functioning and interactions with hepatic, renal, and other systems. The rapid conversions of P piscicida among life cycle stages suggest tightly regulated and efficient developmental mechanisms. We plan to examine whether these mechanisms involve transcriptional regulation or rapid release of stored effectors, in ongoing research with the ultimate goal of designing molecular mechanisms to control toxin production in this organism.

Most dinoflagellate toxins are known to be confined within the cells as endotoxins, freed when cell walls are ruptured by abrasion (e.g., wave action breaking cells of Gymnodinium breve Davis; Steidinger & Baden, 1984) or chemical processes (e.g., digestion of toxic Alexandrium spp. in shellfish gut tracts; Shumway, 1990). Humans become exposed to the toxins by inhaling aerosols with broken cells (G. breve; Steidinger & Baden, 1984), or by consuming contaminated shellfish that concentrated the toxins by filter-feeding the dinoflagellates (Shumway, 1990). In contrast, P piscicida produces highly lipophilic exotoxin(s); hence, the cells apparently carry only residual toxicity (Y. Shimizu, University of Rhode Island, personal communication), but the water and aerosols from the water are toxic. The apparent toxin transport by air as well as water (as mycells) presents new concerns about potential exposure of fishermen who frequent fish kill areas.

Comparison of the three laboratory exposure cases that have been medically evaluated and/or reviewed by co-author D. E. Schmechel provides insights in considering potential human health effects of toxins or sensitizing substances from cultures of P piscicida. Symptoms shared in these exposures and previously described for ciguatera (reef-fish poisoning) or for domoic acid exposure include headache, abdominal cramps or pain, nausea, and vomiting. Like domoic acid, exposure to R piscicida is associated with immediate prolonged abnormalities of short-term memory, but the neurological sequeilae seem less severe for Pfiesteria-related exposures in that the short-term memory dysfunction has been reversible. Unlike ciguatera and domoic acid poisoning, there was no associated diarrhea (Teitelbaum et al., 1990); unlike domoic acid poisoning, there were

no seizures, myoclonus, or motor involvement.

The most obvious difference with neurovisceral ciguatera and domoic acid poisoning (Perl et al., 1990; Swift & Swift, 1993) is that Pficsteria exposure can occur directly through skin contact or inhalation of aerosols from contaminated water and does not require ingestion of seafood or the organism. This suggests that the highly lipophilic toxin(s) may represent extreme health hazards when absorbed. Moreover, ciguatera and domoic acid poisoning usually involve some delay (hours) prior to onset of symptoms, whereas Pfiesteria culture-related exposures resulted in some almost-immediate symptoms. In three cases, residual effects that have occurred after vigorous exercise years after exposure are similar in that there is a delay period (hours) between the exercise and the onset of symptoms (although exercise-related symptomatology differs slightly). Two of the Pfiesteria cases involved some sensory disturbance as part of the clinical syndrome, but in both cases there was minimal peripheral nerve involvement as indicated by clinical examination or EMG/NCV studies, which contrasts with the prominence of paresthesias and sensory reversal phenomena in ciguatera poisoning and the involvement of peripheral nerve.

Other symptoms "unique" to Pfiesteria exposures in comparison to ciguatera poisoning include rapid onset of prominent eye irritation with uniform reddening, respiratory distress, and skin lesions. These symptoms were accompanied by narcosis and altered mental state that rapidly subsided post-exposure, but which were followed by short-term memory loss lasting 1-8 wk. Altered host immunity was suggested in two cases by easy infections and persistent skin lesions (Case B), and recurring respiratory infections and depressed T-cell counts (Case C). The involvement of skin and nervous system in humans mimics the symptomatology of fish poisoned by toxin(s) from P piscicida. Given the severity of the initial syndromes, the three cases of repeated subacute and acute laboratory exposure have shown remarkable recovery. However, significant residual syndromes have persisted 1-6 yr post-exposure as exercise-induced sensory symptoms (Case A); exercise-induced asthenia, "foggy episodes," depressed reading ability, sudden rages, and residual transient sensory symptoms with severe headaches following social use of alcohol (Case B); and exercise-induced asthenia, difficulty in breathing, and markedly increased asthmatic bronchitis and respiratory infections (Case C). Residual and often severe sensory symptoms are also commonly reported by ciguatera victims for years after exposure. In contrast, in cases where transient symptoms followed subacute exposures, cessation of contact with toxic cultures apparently was associated with resolution of symptoms.

On the basis of verbal reports from laypersons, supported by knowledge of some rapid effects of dilute P piscicida cultures on laboratory personnel, we believe there is high likelihood that persons who freqtientisitei of toxic outbreaks by this dinoflagellate in the natural environment have experienced exposure syndromes. Putative cases are being examined by medical review, and careful medical and epidemiological studies are planned. Anecdotal reports to several of the authors from more than 25 local fishermen who frequent sites linked to toxic outbreaks of this dinoflagellate—with descriptions of skin lesions that heal poorly and do not respond to most antibiotics, chronic flu-like symptoms suggesting compromised immune system, and "foggy episodes"—seem analogous to features of laboratory exposure cases. The carefully interviewed case of the shrimp fisherman is highly suggestive of a marine toxin poisoning with onset of symptoms prior to (i.e., unrelated to) ingestion of seafood. Assessment of putative exposures will require careful medical examination, since there may be variation of clinical syndromes depending on route of exposure, dose, and host characteristics. Two of the laboratory exposure cases suggest that significant long-term, postexposure health effects such as suppressed immune system dysfunction and significant neurasthemia may be encountered. However, the role of P piscicida in adverse health impacts stemming from immune system suppression may be masked by the onset of other illnesses or secondary events. Exclusion of other marine toxins, concurrent illnesses, or other medical conditions will be required. Further evaluation will be aided by animal studies in mammals (e.g., the domoic acid model; Perl et al., 1990), isolation of the toxin(s), careful epidemiologic study of potentially exposed populations, and development of methods for assaying toxin levels in water, sea life, and humans.

REFERENCES

Bovee, E. C., and Sawyer, T. K. 1979, Marine Flora and Fauna of the Northeastern United States. Protozoa: Sarcodina: Amoebae, pp. 1-57. NOAA Technical Report NMFS Circular 419. Washington, DC: United States Department of Commerce, NOAA NMFS.

Burkholder, J. M., and Glasgow, H. B., Jr. 1995. Interactions of a toxic estuarine dinoflagellate with microbial predators and prey. Archly. Protistenk, 145:177-188.

Burkholder, J. M., and Sheath, R. G. 1985. Characteristics of softwater streams in Rhode Island. I. A comparative analysis of physical and chemical variables. Hydrobiologia 128:97-108.

Burkholder, J. M., and Wetzel, R. G. 1989. Epiphytic microalgae on natural substrata in a hardwater lake: Seasonal dynamics of community structure, biomass and ATP content, Arch. Hydrobiol. Sum,. 83:1-56.

Burkholder, 1. M., Noga, E. j., Hobbs, C. W., Glasgow, H. B., Jr., and Smith, S. A. 1992. New "phantom" dinoflagellate is the causative agent of major. estuarine fish kills. Nature 358:407-410, 360:768.

Burkholder, J. M., Glasgow, H. B.,)r., Noga, E. 1., and Hobbs, C. W. 1993. The Role of a New Toxic Dinoflagellate in Finfish and Shellfish Kills in the Neuse and Pamlico Estuaries, pp. 1-58. Report No. 93-08 to the Albemarle-Pamlico Estuarine Study, Raleigh, N.C.: NC DEHNR and U.S. EPA National Estuarine Program.

Burkholder, J. M., Glasgow, H. B., Jr., and Hobbs, C. W. 1995a. Distribution and environmental conditions for fish kilts linked to a toxic ambush-predator dinoflagellate. Mar. Ecol. Prof. Ser., in press.

Burkholder, J. M., Glasgow, H. B., Jr., and Steidinger, K. A. 1995b. Stage transformations in the complex life cycle of an ichthyotoxic "ambush-predator" dinoflagellate. In Harmful marine algal blooms, eds. P. Lassus, G. Arzul, E. Erard-Le Denn, P. Gentien, and C. Marcaillou-Le Baut, pp. 567-572. Paris: Lavoisier.

Carmichael, W. W. 1994. The toxins of cyanobacteria. Sci. Am. 270:78-86.

Christian, E. E., Boyer, J. N., and Stanley, D. W. 1991. Multi-year distribution patterns of nutrients within the Neuse River Estuary, North Carolina. Mar, Ecol. Frog. Ser. 71:259-274.

Copeland, B. J., Hodson, R. G., and Riggs, S. R. 1984. The Ecology of the Pamlico River, North Carolina: An Estuarine Profile. U.S. Fish & Wildlife Service Report FWS/OBS-82/06. Washington, D.C.: U.S. Fish and Wildlife Service.

Day, R. W., and Quinn, G. P. 1989. Comparison of treatments after an analysis of variance in ecology. Ecol. Monogr. 59:433-463.

Dennison, W. C., Orth, R. 1., Moore, K. A., Stevenson, J. C., Carter, V., Kollar, S., Bergstrom, P. W., and Batiuk, R. A. 1993. Assessing water quality with submersed vegetation. BioScience 43:86-94. Epperley, S. P., and Ross, S. W. 1986. Characterization of the North Carolina Pamlico-Albemarle Estuarine Complex. NOAA Technical Memorandum NMFS-SEFC-175. Washington, D.C.: NOAA.

Gill, J. L. 1978. Design and analysis of experiments in the animal and medical sciences, Vols. 1, 2, and 3. Ames: Iowa State University Press.

Hallegraeff, G. M. 1993. A review of harmful algal blooms and their apparent global increase. Phycologia 32:79-99.

Jaworski, N. A., Groffman, P. M., Keller, A. A., and Prager, J. C. 1992. A watershed nitrogen and phosphorus balance: The tipper Potomac River basin. Estuaries 15:83-95.

Landsberg, J. H., Steidinger, K. A., and Blakesley, B. A. 1995. Fish-killing dinoflagellates in a tropical aquarium. In Harmful marine algal blooms, eds. P. Lassus, G. Arzul, E. Erard-Le Denn, P. Gentien, and C. Marcaillou-Le Baut, pp. 65-70. Paris: Lavoisier.

Levine, J. F., Hawkins, J. H., Dykstra, M. J., Noga, E. 1., Moye, D. W., and Cone, R. S. 1990. Species distribution of ulcerative lesions on finfish in the Tar-Pamlico River Estuary, North Carolina. Dis. Aquat. Org. 8:1-5.

Mallin, M. A. 1994. Phytoplankton ecology of North Carolina estuaries. Estuaries 17:561-574.

Miller, K. H., Camp, J., Bland, R. W., Hawkins, J. H. III, Tyndall, C. R., and Adams, B. L. 1990. Pamlico Environmental Response Team Report (June-Sept. 1988) Wilmington, N.C.: NC DEHNR.

National Oceanic & Atmospheric Administration. 1992. Status of Fishery Resources off the Southeastern United States for 1991. NOAA Technical Memorandum NMFS-SEFSC-306. Miami, Fla.: U.S. Department of Commerce, NOAA-NMFS.

Noga, E. J., and Dykstra, M. J. 1986. Oomycete fungi associated with ulcerative mycosis in Atlantic menhaden. J. Fish Dis. 9:47-53.
Noga, E. J., Marquardt, N., and Berkhoff, H. 1989. Water Quality and the Health of Estuarine Fishes. Morehead City, N.C., final report to NC DMF.
Noga, E. J., Wright, J. F., Levine, J. F., Dykstra, M. J., and Hawkins, J. H. 1991. Dermatological diseases affecting fishes of the Tar-Pamlico Estuary, North Carolina. Dis. Awn. Org. 10:87-92.
Noga, E. J., Smith, S. A., Burkholder, J. M., Hobbs, C. W., and Bullis, R. A. 1993. A new ichthyotoxic dinoflagellate: Cause of acute mortality in aquarium fishes. Vet. Rec. 133:96-97.
Noga, E. J., Khoo, L., Stevens, J. B., Fan, Z., and Burkholder, J. M. 1995. Novel toxic dinoflagellate causes epidemic disease in estuarine fish. Mar. Pollut. Bull., in press.
North Carolina Department of Environment, Health and Natural Resources. 1990. Water Quality Progress in North Carolina: 1989-1990 305(b) Report. Raleigh, N.C.: NC DEM.
North Carolina Department of Environment, Health and Natural Resources. 1992. Algal Bloom Reports. Raleigh, N.C.: NC DEM.
Paerl, H, W., Mallin, M. A., Rudek, J., and Bates, P. W. 1990. The Potential for Eutrophication and Nuisance Algal Blooms in the Lower Neuse River Estuary, pp. 1-58. Report. 90-15, Albemarle-Pamlico Estuarine Study. Raleigh, N.C.: NC DEHNR and U.S. EPA Estuarine Research Program.
Parsons, T. R., Maita, Y., and Lalli, C. M. 1985. A manual of chemical and biological methods for seawater analysis. New York: Pergamon.
Perl, R. M., Bedard, L., Kosatsky, R., Hocking, J. C., Todd, E. C. D., and Remis, R. S. 1990. An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. N. Engl. I. Med. 322:1775-1780.
Rudek, J., Paerl, H. W., Mallin, M. A., and Bates, P. W. 1991. Seasonal and hydrological control of phytoplankton nutrient limitation in the Neuse River Estuary, North Carolina. Mar. Ecol. Prog, Ser. 75:133-142.
SAS Institute, Inc. 1987. SAS/STAT guide for personal computers, Version 6. Cary, N.C.: SAS Institute, Inc.
Shumway, S. E. 1990. A review of the effects of algal blooms on shellfish and aquaculture. 1. World Aquacult. Soc. 21:65-104.
Smayda, T. J. 1989. Primary production and the global epidemic of phytoplankton blooms in the sea: A linkage? In Novel phytoplankton blooms, eds. E. M. Cosper, V. M. Bricelj, and E. J. Carpenter, pp. 449-483. Coastal & Estuarine Studies No. 35. New York: Springer-Verlag.
Smayda, T. J. 1992. Global epidemic of noxious phytoplankton blooms and food chain consequences in large ecosystems. In Food chains, models and management of large marine ecosystems, eds. K. Sherman, L. M. Alexander, and B. D. Gold, pp. 275-307. San Francisco: Westview Press.
Smith, S. A., Noga, E. J., and Bullis, R. A. 1988. Mcrtality in Tilapia aurea due to a toxic dinollagellate bloom. Proc. Third Int. Colloq. Pathol. Marine Aquaculture, Gloucester Point, Va., p. 167 (abstr.).
Snieszko, S. F., and Axelrod, H, R. 1976. Diseases of fishes. 5th volume in the series, Environmental stress and fish diseases, eds. G. A. Wedemeyer, F. P. Meyer, and L. Smith. Neptune City, N.J.: T. F. H. Publications.
Stanley, D. H. 1992. Historical Trends-Water Quality and Fisheries: Albemarle-Pamlico Sounds. University of North Carolina Sea Grant CoIle:le Program Publication UNC-SG-92-04. Raleigh: UNC Sea Grant.
Steel, J., ed. 1991. Status and Trends Report of the Albemarle-Pamlico Estuarine Study. Raleigh, N.C.: Albemarle-Pamlico Estuarine Study, NC DEI-IMR and U.S. EPA National Estuarine Program.
Steel, 1., and Spence, 1.. 1995. Ten Crucial Issues Concerning North Carolina's Coastal Water Quality. University of North Carolina Sea Grant Publication UNC-SG-94-05. Raleigh, N.C.: UNC Sea Grant, in press.
Steidinger, K. A. 1993. Some taxonomic and biolo l: aspects of toxic dinoflagellates. In Algal toxins in seafood and drinking water, ed. I. R. Falconer, Nil, 1-28. New York: Academic Press.
Steidinger, K. A., and Baden, D. G. 1984. Toxic I arine dinoflagellates. In Dinoflagellates, ed. D. L. Spencer, pp. 201-206. New York: Academic Press;.
Steidinger, K. A., Truby, E., Garrett, J. K., and Burkh, tder, J. M. 1995. The morphology and cytology of a newly discovered toxic dinoflagellate. In Harm of marine algal blooms, eds. P. Lassus, G. Arzul, E. Erard-Le Denn, P. Gentien, and C. Marcaillou-L 3aut, pp. 83-88. Paris: Lavoisier.
Swift, A. E. B., and Swift, T. R. 1993. Review: Ciguateri Toxicol. 31:1-29.
Teitelbaum, J. S., Zatorre, R. J., Carpenter, S., Gendor D., Evans, A. C., Gjedde, A., and Cashman, N. R. 1990.

Neurological sequelae of domoic acid in toxication due to the ingestion of contaminated mussels. N. Engl. I. Med. 322:1781-1787.
Vollenweider, R. A., ed. 1974. A manual on methods for measuring primary production in aquatic environments, 2nd ed., International Biology Program Handbook 12. Oxford: Blackwell.
White, A. W. 1988. Blooms of toxic algae worldwide: Their effects on fish farming and shellfish resources. Proc. Int. Conf. Impacts of Toxic Algae on Mariculture. Trondheim: Aqua-Nor '87 International Fish Farming Exhibition, pp. 9-14.